Platform: Transcription

1150-Plat
Quantifying the Interaction Between Neighboring Gene Circuits
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In heterologous gene expression, a module with two gene circuits responsible for expression of a foreign gene of interest and selection is integrated into the host cell genome. Although the expression level of the foreign gene is generally understood to be determined by the strength of the promoter immediately upstream, several studies have shown that it varies significantly depending on the site and orientation of genomic integration. In addition, the arrangement of the selection circuit within the module may also affect the gene expression level. To test this possibility, we built a set of heterologous modules where two different gene circuits are placed right next to each other in all possible relative orientations. The activities of these gene circuits can be separately driven by external inducers and simultaneously monitored from fluorescent protein levels. We integrated these modules into the genome of budding yeast and quantitatively measured expression levels of both gene circuits at multiple inducer-level combinations. Our preliminary results show that the adjacent gene circuits interact in a cooperative and reciprocal manner; activation of the first gene circuit enhances the expression level of the second gene circuit and vice versa. Moreover, the degree of enhancement depends on the relative orientation of the gene circuits, highest when the promoters are pointing away from each other. Based on our results, we present a rate-equation based model to highlight potential mechanisms of promoter interaction.

1151-Plat
Visualizing the Transcription Cycle of Endogenous RNA Polymerase II in Single Living Cells
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How efficient is transcription in vivo? Recent fluorescence microscopy studies have begun to confront this question by measuring the binding times of RNA polymerase II (pol II) and other components of the transcription machinery in single living cells. This has revealed an extremely dynamic transcription machine that appears to operate with components in continual flux. However, it remains debatable whether or not these rapid dynamics lead to efficient transcription, mainly because it has been difficult to distinguish different phases of the transcription cycle in vivo. For example, using GFP it is possible to visualize the movement of pol II, but directly distinguishing the uninitiated fraction from the initiated or elongating fractions has not yet been possible. To overcome this difficulty, we have loaded fluorescent antibody fragments (Fab) against unphosphorylated and phosphorylated forms of pol II into living cells containing an inducible tandem gene array. Using this unique system, we are quantifying the accumulation of uninitiated (unphosphorylated), initiated (ser 5 phosphorylated), and elongating (ser 2 phosphorylated) forms of pol II to the gene array after induction. Our observations suggest transcription is quite efficient, with pol II being recruited to activated genes within ~3 minutes time, after which ~70% are initiated within 30 seconds and ~35% proceed to elongation within another 30 seconds. We are currently investigating how these transcription dynamics correlate with histone modification dynamics at the gene array.

1152-Plat
Experimental and Stochastic Model Analysis of the Influence of SIC1, CLN2 and CLB5 Transcriptional Noise on the Timing Regulation of G1/S Transition in S. Cerevisiae Cell-Cycle
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Gene expression results in significant fluctuations in transcriptional and translational abundance among cells of a genetically identical population. Remarkably, strong regulatory mechanisms in Saccharomyces cerevisiae cell-cycle enable its progression despite these fluctuations.
Our work combining modeling and experimental approaches investigates the influence of stochasticity in gene expression, especially in transcription, for timing regulation of the G1/S transition. We focus on Sic1, a cyclin-dependent kinase inhibitor, and its interacting partners, the cyclins Cln2 and Clb5. The interplay of these cell-cycle regulators is critical for precise timing of the G1-phase exit.
We quantify SIC1 transcripts level and transcriptional noise in a cell population via fluorescence microscopy by taking advantage of two highly sensitive single molecule detection methods, the MS2-CP and the Fluorescence in situ Hybridisation (FISH) techniques. We used these data to explore a stochastic model for the G1/S transition timing regulation focusing on the fluctuations of SIC1 transcripts level.
The experimental investigation revealed a distribution between 0 and 10 SIC1 mRNAs per cell and a significant increase of transcripts in the G1-phase. Our model predicts that by producing only a few SIC1 mRNAs, as observed in our experiments, the cell ensures low noise level of Sic1 protein and an exact timing of the S-phase entrance.
We conclude that timing properties in yeast cell-cycle are highly influenced on the molecular level by discrete abundance of transcription products. Essentially, transcription regulation is involved in maintaining robustness in cell-cycle progression.
To obtain a comprehensive understanding of transcription regulation at an interacting-proteins-network level, we are now measuring CLN2 and CLB5 transcript levels to investigate the correlation of their transcript levels as well as that of SIC1.

1153-Plat
Single Molecule Probing of the Human Tumor Suppression Transcription Initiation Machinery
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In humans, transcription Pre-Initiation Complex (PIC) assembly begins with the recognition of the core promoter by the large multi-subunit TFIID complex and culminates with recruitment of RNA Polymerase II. Transcriptional activators, such as the tumor suppressor p53 protein, stimulate transcription initiation, in part by binding sequences upstream of the promoter and help recruit TFIID to core promoter DNA.
In response to cellular stress the tumor suppressor p53 protein becomes activated and turns on a variety of target genes in a highly coordinated manner to dictate cellular fate. Cellular levels of p53 are essential in dictating gene expression programs based upon the affinity of p53 for binding sites upstream of the core promoter. Many general transcription factors that bind to the core promoter are known to interact with p53 and potentially alter the affinity of each factor for the promoter DNA. Despite almost 20 years of research, the dynamic interplay between p53 and components within the general transcription machinery at physiological promoters remains poorly understood.
We have probed in real-time the association between human p53, TFIID, and various p53 target gene promoters, using a combination of in vitro single molecule tethered particle motion(TPM) and high-resolution TIRF co-localization assays. Our results show that p53 and TFIID act cooperatively to aid in recruitment to promoter DNA. In addition, differences in cooperative assembly of p53 and TFIID on various physiological promoters underscore a mechanism for p53's graded response to cellular stress. TPM analysis reveals p53 and TFIID dependent DNA looping suggesting stable interactions between upstream bound activators and core promoter bound factors. We have further examined the influence of additional general transcription factors on p53 mediated transcription complex assembly.

1154-Plat
Single-Molecule Insights on the Human RNA Polymerase II Transcription Regulation: Assembly of the Pre-Initiation Complex, Re-Initiation Scaffold, and the Roles of Activators
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Transcription of all protein-coding genes in human cells begins with the assembly of the RNA polymerase II pre-initiation complex (PIC) composed of more than forty polypeptides (total size of ~ 3MDa). Due to the high complexity and the dynamic nature of the PIC, the mechanism of its assembly and regulation remains elusive after decades of conventional biochemical studies.
We have developed a surface-based, promoter-specific Pol II transcription system suitable for single-molecule work. The system consists of (a) an imaging surface and a fluidics system supporting Pol II transcription from immobilized DNA templates; (b) site-specific labeling of recombinant and complex transcription factors; and (c) novel probes capable of single-molecule, real-time

detection of mRNA synthesis. We have simultaneously imaged thousands of single-molecule transcription events at sub-second time resolution for hour-long time periods with two separate multi-color fluorescence imaging techniques: (a) actively stabilized (drift of <1 nm over hours) temperature-controlled total internal reflection (TIR) microscopy; and (b) the zero mode waveguide multiplex confocal imaging system developed by Pacific Biosciences.
With this experimental setup, we have observed up to eight rounds of promoter-specific Pol II transcription per DNA template. The efficiency of transcription re-initiation was found much higher than the efficiency of the first transcription round, suggesting a ''scaffold'' left from the first round could facilitate re-initiation. We have further found that a key component of this scaffold is the promoter recognizing TFIID complex. Using multiple fluorescently labeled GTFs (TFIIB TFIID, TFIIF, Pol II, and TFIIE) we are currently investigating the structure and regulation of this re-initiation scaffold, the order of the PIC assembly, and the mechanism of transcription modulation by sequence-specific activators in the context of promoter DNA elements.

1155-Plat
Super-Resolution Fluorescence Microscopy of Transcription Sites in E. Coli
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Bacteria have long been thought of as 'bags of enzymes' lacking internal structure. However recent studies have shown that many of their essential processes, including transcription, are spatially organized [1]. Still, the precise distribution of active RNA polymerases (RNAPs) remains unclear, as the main tool for studying protein localization - diffraction-limited fluorescence microscopy - has been unable to resolve important structural details.
We have investigated the spatial organization of RNAP in E. coli using the super-resolution technique photoactivation-localization microscopy (PALM) [2]. After tagging the endogenous RNAP population with a fluorescent protein, we imaged its distribution under various growth conditions. While its distribution was somewhat random in cells grown in minimal media, we found strong clustering and 'banding' in cells grown in rich media. We have applied various strategies to analyze such clustering in an effort to understand its underlying biological significance.
References
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1156-Plat
Protein-Level Fluctuation Correlation at the Microcolony Level and its Application to the Vibrio Harveyi Quorum-Sensing Circuit
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Gene expression is stochastic, and noise that arises from the stochastic nature of biochemical reactions propagates through active regulatory links. Thus, correlations in gene-expression noise can provide information about regulatory links. We present what to our knowledge is a new approach to measure and interpret such correlated fluctuations at the level of single microcolonies, which derive from single cells. We demonstrated this approach mathematically using stochastic modeling, and applied it to experimental time-lapse fluorescence microscopy data. Specifically, we investigated the relationships among LuxO, LuxR, and the small regulatory RNA qrr4 in the model quorum-sensing bacterium Vibrio harveyi. Our results show that LuxR positively regulates the qrr4 promoter. Under our conditions, we find that qrr regulation weakly depends on total LuxO levels and that LuxO autorepression is saturated. We also find evidence that the fluctuations in LuxO levels are dominated by intrinsic noise. We furthermore propose LuxO and LuxR interact at all autoinducer levels via an unknown mechanism. Of importance, our new method of evaluating correlations at the microcolony level is unaffected by partition noise at cell division. Moreover, the method is first-order accurate and requires less effort for data analysis than single-cell-based approaches. This new correlation approach can be applied to other systems to aid analysis of gene regulatory circuits. This work has been published in Biophys J. 100, 3045-53 (2011).

1157-Plat
Quantitative Dissection of RNA Polymerase-Promoter Interactions using Protein Beacon Assay
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Promoter recognition and melting by RNA polymerase (RNAP) are key points in gene expression and regulation. E. coli RNAP binding to promoter DNA and model promoter fragments can be measured using a new protein beacon assay. The assay relies on the detection of fluorescence signal from a fluorescent label incorporated into the σ70 subunit of RNAP close to region 2.3 of σ70, part of RNAP that recognizes the −10 promoter element. The ground level fluorescence of such RNAP beacon is low because the region 2.3 aromatic amino acids quench the fluorescence. When RNAP beacon binds promoter, the quenching interactions become destroyed, leading to increased fluorescence.
Promoter melting in bacteria is nucleated at upstream edge of the transcription bubble. The mechanism of downstream propagation of the transcription bubble to include the transcription start site is unclear. We introduced new downstream fork junction promoter fragments mimicking the downstream segment of promoter complexes. We demonstrated that RNAP binding to downstream fork junctions was coupled with DNA melting around the transcription start point and identified structural determinants of affinity and transcription activity of RNAP-downstream fork junction complexes.
The product of E. coli T7 bacteriophage gene 2 (gp2 protein) is a potent inhibitor of host RNAP. We applied the beacon assay to the mechanism of gp2 inhibition. We measured the effect of gp2 on RNAP binding to various promoter fragments. In this way, the effect of gp2 on RNAP-promoter interactions was dissected. Gp2 greatly decreased RNAP affinity to downstream promoter duplex and inhibited RNAP binding to template and non-template strand segments located between the −10 promoter element and downstream edge of the transcription bubble. The inhibition of RNAP interactions with the transcription bubble by gp2 is a novel effect, which may occur via allosteric mechanism.

Platform: Muscle Regulation

1158-Plat
Visualization of Troponin on Muscle Thin Filaments by Single Particle Analysis
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Troponin on the thin filaments of striated muscle couples Ca-concentration changes to movement of tropomyosin. Ca-free troponin is thought to shift tropomyosin to the myosin-blocking position, a constraint that is released after Ca binds. Although the location and regulatory movements of tropomyosin have been defined at near-atomic resolution, the organization of troponin on thin filaments has not been determined definitively, and different models of troponin position on actin are contradictory. Here novel single-particle analysis (SPA) protocols were designed to reconstruct thin filament structure from electron micrographs of negatively-stained cardiac muscle thin filaments at low-Ca. Troponin-tropomyosin has a tendency to dissociate from filaments under EM conditions, and only filaments showing evidence of bound troponin were chosen for analysis. The axial location of troponin densities on actin was identified by newly developed algorithms. Filament polarity was determined according to Narita and Maeda (2007). Filaments were divided computationally into 46.8 nm particles centered on troponin. SPA was carried out on these particles without applying helical averaging, and successively refined reconstructions did not degrade the troponin-tropomyosin signal. Tropomyosin strands extended smoothly and continuously over adjacent actin monomers along the long-pitch filament helix, occupying the myosin blocking position. Tropomyosin atomic models fitted well to corresponding densities close to the binding site on actin described in Li et al. (2011) for troponin-free filaments. The troponin core-domain position and orientation deviated slightly from those proposed by Pirani et al. (2006). A broadened tropomyosin strand density found on the barbed-end side of the troponin core-domain appeared to bifurcate to link tropomyosin and the core-domain. We attribute this density to the tail of troponin-T, whose location corroborates the polarity of troponin on actin proposed by Flicker et al. (1982).